

# Translating intracellular calcium signaling into models

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## Abstract

The rich experimental data on intracellular calcium has put theoreticians in an ideal position to derive models of intracellular calcium signaling. Over the last 25 years, a large number of modeling frameworks have been suggested. Here, I will review some of the milestones of intracellular calcium modeling with a special emphasis on calcium-induced-calcium release (CICR) through inositol-1,4,5-trisphosphate and ryanodine receptors. I will highlight key features of CICR and how they are represented in models as well as the challenges that theoreticians face when translating our current understanding of calcium signals into equations. The selected examples demonstrate that a successful model provides mechanistic insights into the molecular machinery of the  $\text{Ca}^{2+}$  signaling toolbox and determines the contribution of local  $\text{Ca}^{2+}$  release to global  $\text{Ca}^{2+}$  patterns, which at the moment cannot be resolved experimentally. The protocols in this chapter provide introductory examples to modeling CICR, which may serve as a starting point for theoretically exploring the wealth of intracellular calcium signals and link it to experimental data.

## Introduction

One of the most fascinating features of calcium ( $\text{Ca}^{2+}$ ) as a second messenger is its versatility (Berridge et al. 2000). Almost every cell type shows  $\text{Ca}^{2+}$  signals, and even within a single cell the number of signaling pathways that involve  $\text{Ca}^{2+}$  is huge. From a modeler's perspective the broad spectrum of interactions renders  $\text{Ca}^{2+}$  an intriguing yet challenging study object. The fascination originates from the large dynamic repertoire of  $\text{Ca}^{2+}$  signals. Most  $\text{Ca}^{2+}$  responses begin with the elevation of the cytosolic  $\text{Ca}^{2+}$  concentration through either  $\text{Ca}^{2+}$  entry from the extracellular space or  $\text{Ca}^{2+}$  liberation from intracellular organelles such as the endoplasmic or sarcoplasmic reticulum (ER/SR). Although the molecular details of the ion channels that are responsible for the increase in the cytosolic  $\text{Ca}^{2+}$  concentration differ, in all cases,  $\text{Ca}^{2+}$  forms a plume of high concentration around the site of influx just after a channel opens. These microdomains form the smallest functional unit of intracellular  $\text{Ca}^{2+}$  signals (Berridge 2006) from which larger  $\text{Ca}^{2+}$  patterns are formed. For example, it is the orchestrated action of microdomains that gives rise to cellular responses such as  $\text{Ca}^{2+}$  waves and  $\text{Ca}^{2+}$  oscillations (Bootman et al. 1997). Some  $\text{Ca}^{2+}$  waves travel through the entire cell, while others only spread through parts of the

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cytoplasm resulting in abortive waves. The existence of  $\text{Ca}^{2+}$  microdomains and  $\text{Ca}^{2+}$  waves already points to a defining characteristic of intracellular  $\text{Ca}^{2+}$  signals – they vary largely in their temporal duration and their spatial spread. On the temporal scale, intracellular  $\text{Ca}^{2+}$  signals range from events faster than microseconds (binding and unbinding of  $\text{Ca}^{2+}$  to target molecules), to cellular  $\text{Ca}^{2+}$  transients that last minutes ( $\text{Ca}^{2+}$  waves and oscillations). At the same time, intracellular  $\text{Ca}^{2+}$  operates on length scales from a few nanometers (molecular binding sites) up to hundreds of micrometers ( $\text{Ca}^{2+}$  waves). The challenge for modelers arises from the realization that a complete account of intracellular  $\text{Ca}^{2+}$  requires us to incorporate the entire spatio-temporal spread, i.e. more than eight orders of magnitude in space and over six orders of magnitude in time.

The large range of length and time scales of intracellular  $\text{Ca}^{2+}$  patterns has led to various modeling approaches. A common research practice has been to focus on one class of  $\text{Ca}^{2+}$  signals at a time. For example, detailed investigations of the inositol-1,4,5-trisphosphate ( $\text{IP}_3$ ) receptor ( $\text{IP}_3\text{R}$ ) have been conducted that have greatly facilitated our mechanistic understanding of localized  $\text{Ca}^{2+}$  release events such as  $\text{Ca}^{2+}$  blips and puffs (Thul and Falcke 2004a; Shuai et al. 2007; Rüdiger et al. 2007; Taufiq-Ur-Rahman et al. 2009; Thul et al. 2009b; Swaminathan et al. 2009). More recently, a number of studies have addressed the interaction between different levels of the  $\text{Ca}^{2+}$  signaling hierarchy in a three dimensional cellular environment. To cope with the increased computational demand of simulating three spatial dimensions, all approaches had to introduce approximations. For instance, the coupling between  $\text{IP}_3\text{R}$  clusters with a detailed stochastic gating scheme for the receptor was achieved at the cost of a small number of  $\text{IP}_3\text{R}$  clusters and an idealized spherical cellular geometry (Skupin and Falcke 2009; Skupin et al. 2010; Thurley and Falcke 2011). In a study of an atrial myocytes (Thul et al. 2012), the authors employed a realistic distribution of ryanodine receptor ( $\text{RyR}$ ) clusters but a simplified threshold dynamics for  $\text{Ca}^{2+}$  liberation. By focusing on a small number of  $\text{RyR}$  clusters in a cardiac myocyte, Izu *et al.* could incorporate detailed stochastic dynamics for  $\text{RyRs}$  (Izu et al. 2006). A combination of a small number of  $\text{IP}_3\text{R}$  clusters and an approximation of the  $\text{Ca}^{2+}$  concentration profile around a cluster was studied in Solovey *et al.* (2008). In contrast to the spatially extended models, each  $\text{Ca}^{2+}$  ion is treated separately in a fully stochastic simulation for a point model of a hepatocyte in (Dupont et al. 2008).

In this chapter, I will illustrate modeling concepts and applications with  $\text{Ca}^{2+}$  liberation from the ER or SR through either the  $\text{IP}_3\text{R}$  or the  $\text{RyR}$ . The main motivation for this selection comes from the fact that these receptors present an integral component in the generation of  $\text{Ca}^{2+}$  waves and oscillations, and therefore are vital for mounting a physiological response to extracellular stimuli (Thul et al. 2008a; Dupont et al. 2011; Parekh 2011). It is worth noting that  $\text{IP}_3\text{Rs}$  and  $\text{RyRs}$  differ significantly from each other in their molecular structure, their gating properties and expression patterns (Foskett et al. 2007; Zalk et al. 2007). Different tissues and cell-lines express various isoforms, each tailored to specific signaling needs. Therefore conclusions drawn for the  $\text{IP}_3\text{R}$  cannot necessarily be transferred to the  $\text{RyR}$ .

The modeling studies mentioned above highlight some of the latest developments in a long evolution of  $\text{Ca}^{2+}$  models. In what follows I will describe

three modeling approaches of increasing complexity that are stepping stones towards the more sophisticated modeling frameworks that we use today.

## The well stirred cell

A common and historically an often used assumption is that a cell presents a well-stirred bioreactor. In this setting, the  $\text{Ca}^{2+}$  concentration is the same at all points within each cellular compartment such as the cytosol, the ER/SR or across all mitochondria. From a mathematical point of view, this translates into describing the dynamics of the  $\text{Ca}^{2+}$  concentration in each compartment with an ordinary differential equation (ODE). For example, the rate of change of the cytosolic  $\text{Ca}^{2+}$  concentration ( $c$ ) equals the sum of all  $\text{Ca}^{2+}$  fluxes into the cytoplasm ( $J_{in}$ ) minus the sum of all  $\text{Ca}^{2+}$  fluxes out of the cytosol ( $J_{out}$ ):

$$\frac{dc}{dt} = J_{in} - J_{out}. \quad (1)$$

This simple relation has spawned a large number of cellular  $\text{Ca}^{2+}$  models, most of which differ in the details of the  $\text{Ca}^{2+}$  influx. Assuming that the main contribution to changes in the intracellular  $\text{Ca}^{2+}$  concentration results from the  $\text{IP}_3\text{R}$ , various schemes have been put forward for the receptor as outlined in e.g. (Meyer and Stryer 1988; Goldbeter et al. 1990; De Young and Keizer 1992; Li and Rinzel 1994; Sneyd and Dufour 2002; Mak et al. 2003) and see (Sneyd and Falcke 2005) for a review. The value of all of these  $\text{IP}_3\text{R}$  models is that they provide different mechanistic interpretations of the experimental data sets that they were fitted to. Some of these models assume that  $\text{Ca}^{2+}$  and  $\text{IP}_3$  can associate in any order with their respective binding sites, while other models stipulate a strict sequential binding order or an allosteric transformation to the active state.

Another assumption of such models is that a given value of the cytosolic  $\text{Ca}^{2+}$  concentration results in exactly one fraction of active  $\text{IP}_3\text{Rs}$ , i.e. the  $\text{Ca}^{2+}$  release flux through the  $\text{IP}_3\text{R}$  is completely *deterministic*. However, this is in stark contrast to experiments in e.g. *Xenopus* oocytes that showed the spontaneous emergence of  $\text{Ca}^{2+}$  puffs (Parker and Yao 1996; Sun et al. 1998). To reflect such random initiation of  $\text{Ca}^{2+}$  liberation, modelers replaced the deterministic  $\text{Ca}^{2+}$  flux through the  $\text{IP}_3\text{R}$  with a *stochastic* function. The simplest implementation of this modeling approach is to take any of the deterministic ODE models listed above and change the description of the  $\text{IP}_3\text{R}$   $\text{Ca}^{2+}$  flux to its stochastic counterpart by treating the  $\text{IP}_3\text{R}$  as a Markov chain (Shuai and Jung 2002).

While ODE models of intracellular  $\text{Ca}^{2+}$  dynamics – whether deterministic or stochastic – have proven popular in the past, the richness of new experimental data prompts questions about the validity of this approach. A different interpretation of an ODE representation of the intracellular  $\text{Ca}^{2+}$  dynamics is that the equation describes the average  $\text{Ca}^{2+}$  concentration in the cell. This is indeed a good approximation if the  $\text{Ca}^{2+}$  concentration varies marginally across the cell and hence every point in the cell experiences an almost identical  $\text{Ca}^{2+}$  concentration. However, the presence of spatially localized  $\text{IP}_3\text{R}$  clusters and  $\text{Ca}^{2+}$  microdomains already indicates that the  $\text{Ca}^{2+}$  concentration can change drastically from one part of the cell to another. For example, the cytosolic  $\text{Ca}^{2+}$  concentration rises to more than  $150\mu\text{M}$  at an open  $\text{IP}_3\text{R}$  cluster, while the basal  $\text{Ca}^{2+}$  concentration is around  $50\text{-}150\text{nM}$  (Thul and Falcke 2004a). This represents more than 3 orders of magnitude, and given the sharp gradients of

the  $\text{Ca}^{2+}$  concentration around an active  $\text{IP}_3\text{R}$  cluster, averaging does not capture the true concentration profiles. The issue of largely distinct  $\text{Ca}^{2+}$  concentrations also affects the mechanistic interpretation of ion channel models. Based on thermodynamic principles, the binding rate of  $\text{Ca}^{2+}$  to a designated binding site is proportional to the  $\text{Ca}^{2+}$  concentration. Averaged  $\text{Ca}^{2+}$  concentrations usually peak around  $2\mu\text{M}$ . Since realistic values of the  $\text{Ca}^{2+}$  concentration may exceed the average by a factor of 100, the binding rate is 100 times faster in the latter case than in the former. This has important consequences for processes such as  $\text{Ca}^{2+}$  induced activation or  $\text{Ca}^{2+}$  induced inhibition. Only realistic values of the cellular  $\text{Ca}^{2+}$  concentration can unravel the mechanistic details of  $\text{Ca}^{2+}$  dependent molecules and in turn faithfully represent the cellular  $\text{Ca}^{2+}$  dynamics.

It is worth remembering that models are designed to answer a particular research question and to make predictions. Models should not be limited to reproducing experimental findings. Often, an ODE model for averaged  $\text{Ca}^{2+}$  concentrations provides preliminary insights into the problem at hand and guides researchers towards more sophisticated approaches. From a practical point of view, ODE models frequently serve as a starting point to explore new signaling pathways, partly because there might not be sufficient experimental data to construct a more detailed  $\text{Ca}^{2+}$  model, and partly because the mathematical analysis of ODEs is well established and computationally cheap. Moreover, powerful analytical tools exist that give deeper insights into the mathematical details of such models. Therefore ODE models for the averaged  $\text{Ca}^{2+}$  concentration may prove useful, despite being a poor reflection of the underlying physiology.

## From one compartment to the next

It became apparent early on that for models of cardiac action potentials and for local control models of excitation-contraction coupling that averaged  $\text{Ca}^{2+}$  concentrations across the cell presented a poor description of the cellular environment (DiFrancesco and Noble 1985; Stern 1992). The latter serves as a prime example of how details of the spatial arrangement of  $\text{Ca}^{2+}$  conducting ion channels lead to predictive modeling. To trigger contraction,  $\text{Ca}^{2+}$  enters the cell through L-type voltage dependent  $\text{Ca}^{2+}$  channels (VDCCs) upon depolarization of the plasma membrane. In turn, RyRs open through  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release (CICR) (Bers 2002). A key feature of VDCCs and RyRs is that they often co-localize within a distance of approximately 15nm, hence providing RyRs with a privileged access to the  $\text{Ca}^{2+}$  influx through VDCCs. It is this localized  $\text{Ca}^{2+}$  entry that controls CICR, not a whole cell averaged L-type  $\text{Ca}^{2+}$  current. Indeed, excitation-contraction coupling can only be explained through a spatially non-uniform distribution of VDCCs-RyRs clusters (Soeller and Cannell 2004; Cannell and Kong 2012). Such spatial heterogeneity is also at the heart of the model by DiFrancesco and Noble (DiFrancesco and Noble 1985). To reproduce experimental time courses of the intracellular  $\text{Ca}^{2+}$  concentration, the authors had to assume two compartments within the SR: one pool that resequesters  $\text{Ca}^{2+}$  from the cytosol and one pool that releases  $\text{Ca}^{2+}$  into the cytosol. This highlights what is known as compartmentalized modeling. In Stern's model the two conceptual compartments are the dyadic cleft and the bulk cytosol, while DiFrancesco and Noble distinguish between the junctional and non-junctional SR

as well as the bulk cytosol. Note that compartmentalized models differ from whole cell models introduced in the previous section because they allow the division of morphologically continuous cellular compartments into functional subspaces (e.g. junctional and non-junctional SR). In turn, this leads to spatially varying  $\text{Ca}^{2+}$  concentrations as opposed to spatially homogenous  $\text{Ca}^{2+}$  concentrations in the well-stirred cell. From a mathematical point of view, compartmentalized models take on the form of coupled ODEs between the different compartments. For example, the model in (DiFrancesco and Noble 1985) can be written as

$$\frac{dc}{dt} = (J_{ion} + J_{rel} - J_{up})/V_i, \quad \frac{dc_{up}}{dt} = (J_{up} - J_{tr})/V_{up}, \quad \frac{dc_{rel}}{dt} = (J_{tr} - J_{rel})/V_{rel},$$

where  $c$ ,  $c_{up}$  and  $c_{rel}$  denote the  $\text{Ca}^{2+}$  concentration in the cytosol, the uptake pool and the release pool of the SR, respectively, with corresponding volumes  $V_i$ ,  $V_{up}$  and  $V_{rel}$ . The three concentrations change due to the ionic flux  $J_{ion}$  across the plasma membrane, the release flux  $J_{rel}$  from the release pool into the cytosol, the uptake flux  $J_{up}$  from the cytosol into the uptake pool and the transfer flux  $J_{tr}$  from the uptake compartment to the release compartment.

The challenge for modelers lies in finding descriptions for all these fluxes, which often reflect mechanistic insights into the underlying dynamics. In this respect, compartmentalized models demand the same input as ODE models for averaged  $\text{Ca}^{2+}$  concentrations. One major difference, however, is that some of the fluxes that are assumed to be present across the entire cell in models for the averaged  $\text{Ca}^{2+}$  concentration only occur for certain compartments. For example, the uptake current is usually assumed to operate throughout a well-stirred cell, but is restricted to the uptake compartment in (DiFrancesco and Noble 1985). From a mechanistic point of view, the uptake current is often carried by sarco-endoplasmic  $\text{Ca}^{2+}$  ATPase (SERCA) pumps and can be described by

$$J_{up} = v_m \frac{c^2}{K^2 + c^2},$$

where  $v_m$  and  $K$  represent the maximal uptake current and the value of the  $\text{Ca}^{2+}$  concentration at which pumps work at half their maximal uptake rate (EC50), respectively. The above equation assumes that the  $\text{Ca}^{2+}$  flux is unidirectional and only determined by the cytosolic  $\text{Ca}^{2+}$  concentration. However, experimental measurements strongly suggest that firstly luminal  $\text{Ca}^{2+}$  feeds back to SERCA pump dynamics and secondly  $\text{Ca}^{2+}$  movement between the cytosol and the ER/SR proceeds along multiple distinct states of the SERCA pump molecule. These experimental results have led to more comprehensive models of SERCA pumps such as in (Sneyd et al. 2003; Shannon et al. 2004; Higgins et al. 2006; Koivumäki et al. 2009; Tran et al. 2009)

The advantage of compartmentalized models lies in the computational ease in accounting for largely varying  $\text{Ca}^{2+}$  concentrations and spatial gradients. A single point model cannot incorporate the different peak values and time courses of e.g. the subsarcolemmal and bulk  $\text{Ca}^{2+}$  concentration in cardiac myocytes. On the other hand, a two-compartment model accomplishes this easily.

Compartmentalized models have significantly advanced our understanding of  $\text{Ca}^{2+}$  handling and the interaction between the membrane potential and the intracellular  $\text{Ca}^{2+}$  concentration in cardiac myocytes (Shiferaw et al. 2003; Shannon et al. 2004; Shiferaw and Karma 2006; Koivumäki et al. 2011). However, deriving multi-compartment descriptions for intracellular  $\text{Ca}^{2+}$

signaling suffers from two major shortcomings. Firstly, it is not clear a priori how many compartments are needed to account for experimental measurements. Different models use different numbers of compartments and yet describe the same physiology (see e.g. table 1 in (Fink et al. 2011)). Secondly, compartmentalized models depend on the volumes of the compartments. It might appear straightforward to estimate the volume of the dyadic cleft, but it is less intuitive where to draw the line between the junctional and non-junctional SR or the bulk and the subsarcolemmal cytosolic space. The last point illustrates the fact that some of the compartments do not correspond to actual physical entities but to functional units that organize  $\text{Ca}^{2+}$  handling. Moreover, the spatial extent of these functional compartments may change over time due to molecular interactions, which in turn renders the volume estimation difficult to make. A prime example for this scenario is the impact of buffers on  $\text{Ca}^{2+}$  release through  $\text{IP}_3$  channels. Especially mobile buffers can increase the effective size of a microdomain around an open  $\text{IP}_3\text{R}$  cluster.  $\text{Ca}^{2+}$  buffers are molecules that bind  $\text{Ca}^{2+}$  and hence increase the cellular  $\text{Ca}^{2+}$  capacity. It is worth noting that more than 90% of cellular  $\text{Ca}^{2+}$  is buffered under normal conditions and that buffers significantly affect local and global  $\text{Ca}^{2+}$  signals (Keener and Sneyd 2001; Falcke 2003a; Dargan and Parker 2003; Zeller et al. 2009). In addition, varying the number of  $\text{IP}_3\text{Rs}$  in a cluster changes the functional volume and hence the dynamics of compartmentalized  $\text{IP}_3\text{R}$  clusters such as modeled in (Williams et al. 2008).

The conceptual simplicity of compartmentalized models makes them ideal candidates to start exploring cellular heterogeneity with low computational demand. However, no matter how many compartments are used and how sophisticated they are, any compartmentalized model suffers from the above shortcomings. The only way to circumvent these issues is to treat the cellular space as what it is: a continuous representation of a cell where the only boundaries are those of the plasma membrane and intracellular organelles. In the next section, we will examine some more realistic models of intracellular  $\text{Ca}^{2+}$  dynamics. However, a better representation of the physiological reality comes at a price. Larger computational overheads are required and more involved mathematical analysis is needed, if it is feasible at all.

## Spatially extended cell models

$\text{Ca}^{2+}$  waves, whether spontaneous or triggered, abortive or cell-wide, correspond to one of the most common forms of  $\text{Ca}^{2+}$  signals. To fully map the large dynamic repertoire of  $\text{Ca}^{2+}$  waves, modelers have to go beyond the framework of ODEs discussed so far and turn to partial differential equations (PDEs). In contrast to the ODEs discussed above where the  $\text{Ca}^{2+}$  concentration only depends on time, PDEs treat the  $\text{Ca}^{2+}$  concentration as dependent on both space  $x$  and time  $t$ , i.e.  $c = c(x, t)$ . In its simplest form, the spatio-temporal evolution of the  $\text{Ca}^{2+}$  concentration is captured by

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2} + f(c), \quad (2)$$

where  $D$  denotes the (effective) diffusion coefficient of  $\text{Ca}^{2+}$  in the cytosol and  $f$  describes the local  $\text{Ca}^{2+}$  dynamics, i.e.  $\text{Ca}^{2+}$  release through  $\text{IP}_3\text{Rs}$  and  $\text{RyRs}$  or  $\text{Ca}^{2+}$  resequestration by SERCA pumps from the cytosol to the ER or SR. In this

respect,  $\text{Ca}^{2+}$  dynamics, as modeled by PDEs, has a direct connection to the ODEs outlined above, since the function  $f$  in equation (2) is the same as the right hand side of equation (1). The effective diffusion coefficient reflects the impact of  $\text{Ca}^{2+}$  buffers on  $\text{Ca}^{2+}$  transport. Essentially,  $\text{Ca}^{2+}$  diffuses through the cytosol either as a free ion or bound to mobile buffers. Since buffers are much larger than  $\text{Ca}^{2+}$ , buffer-bound  $\text{Ca}^{2+}$  diffuses more slowly than free  $\text{Ca}^{2+}$ . An effective diffusion coefficient accounts for these different transport velocities. One method to compute  $D$  is the fast buffer approximation (Wagner and Keizer 1994). To date,  $\text{Ca}^{2+}$  waves have been studied in great detail, and I refer the reader to (Sneyd and Tsaneva-Atanasova 2002; Falcke 2004) for recent reviews on  $\text{Ca}^{2+}$  wave propagation. Here, I would like to focus on some selected aspects of  $\text{Ca}^{2+}$  waves.

The main driving force behind  $\text{Ca}^{2+}$  waves is CICR. Suppose  $\text{Ca}^{2+}$  is liberated at one  $\text{Ca}^{2+}$  release site while all neighboring  $\text{Ca}^{2+}$  release sites are quiescent. Calcium then diffuses from the active  $\text{Ca}^{2+}$  release site to the dormant sites, which increases the probability of these channels to open. Once these channels open,  $\text{Ca}^{2+}$  is liberated and in turn diffuses to adjacent  $\text{Ca}^{2+}$  release sites where a new round of  $\text{Ca}^{2+}$  liberation is triggered. In this way, a saltatory  $\text{Ca}^{2+}$  wave propagates through a cell. For  $\text{IP}_3\text{Rs}$ , the notion of CICR is often illustrated by the bell-shaped dependence of the stationary open probability of the  $\text{IP}_3\text{R}$  on the cytosolic  $\text{Ca}^{2+}$  concentration (Bezprozvanny et al. 1991; Foskett et al. 2007). A small rise of the cytosolic  $\text{Ca}^{2+}$  concentration above base level leads to a significant increase in the  $\text{IP}_3\text{R}$  open probability. However, steady-state data do not necessarily capture the true dynamics of an  $\text{IP}_3\text{R}$ , neither is a bell-shaped dependence of the open probability necessary to explain observed  $\text{Ca}^{2+}$  signals (Sneyd and Falcke 2005). As a consequence, it is more appropriate to conceptualize CICR as  $\text{Ca}^{2+}$  excitability (Keizer et al. 1995). Borrowing ideas from nonlinear dynamical systems,  $\text{Ca}^{2+}$  excitability refers to the fact that  $\text{Ca}^{2+}$  liberation is only initiated at a cluster of closed  $\text{Ca}^{2+}$  releasing channels if the cluster state is sufficiently perturbed. One such perturbation is the increase in the cytosolic  $\text{Ca}^{2+}$  concentration, others include a rise of the  $\text{IP}_3$  concentration or phosphorylation of the receptor molecules. One class of models for  $\text{Ca}^{2+}$  wave propagation that builds on the notion of a critical value of the cytosolic  $\text{Ca}^{2+}$  concentration to trigger  $\text{Ca}^{2+}$  release are those of the fire-diffuse-fire (FDF) type (Pearson and Ponce-Dawson 1998; Keizer et al. 1998; Dawson et al. 1999). Also known as threshold models,  $\text{Ca}^{2+}$  liberation starts as soon as the cytosolic  $\text{Ca}^{2+}$  concentration reaches a critical value. Calcium release continues for a fixed duration, comparable to the lifetime of a  $\text{Ca}^{2+}$  puff or spark, then the release site closes and becomes refractory. The beauty of FDF models is that they are amenable to a rigorous mathematical analysis and computationally inexpensive. The first property makes them ideal candidates to study large portions of parameter space since expressions for key features of traveling waves such as the wave speed are available in closed form. Instead of running a large number of simulations all that is needed is to evaluate analytical expressions, which can be done in a fraction of the time that is required for the numerical simulations. For example, the impact of SERCA pumps on  $\text{Ca}^{2+}$  wave propagation has been studied in (Coombes 2001), and investigating the interplay between the cytosol and the SR provided explanations for two novel wave types: tango waves (Li 2003; Thul et al. 2008b) and sensitization waves (Keller et al. 2007; Thul et al. 2009a). While deterministic models such as the original FDF description are still instrumental



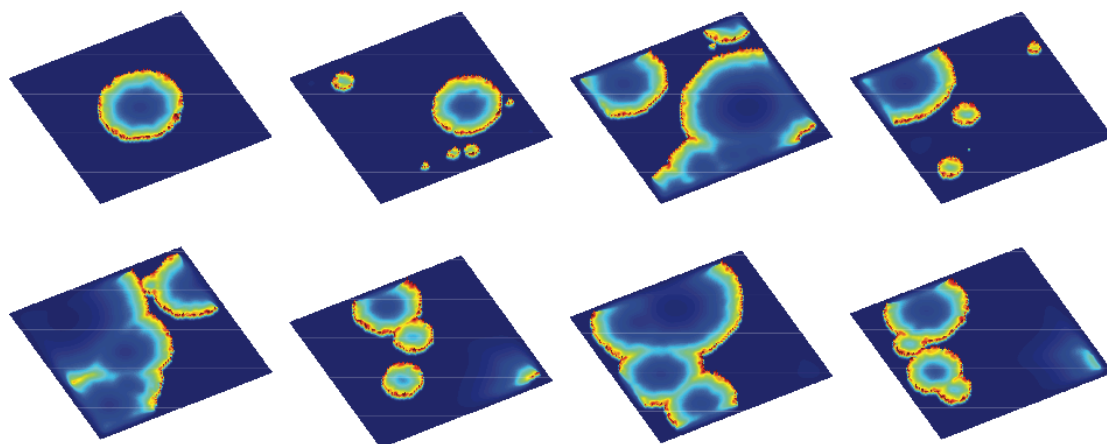
in advancing our understanding of intracellular  $\text{Ca}^{2+}$  waves, hybrid frameworks that incorporate the stochastic nature of  $\text{Ca}^{2+}$  release have gained considerable attention. In this respect, threshold models are ideally suited to capture the random opening of  $\text{IP}_3\text{Rs}$  and  $\text{RyRs}$ , and study stochastic  $\text{Ca}^{2+}$  waves. In these models, the constant threshold for  $\text{Ca}^{2+}$  liberation is replaced by a fluctuating value which can in principle be derived from experiments (Izu et al. 2001; Coombes and Timofeeva 2003).

The above examples for  $\text{Ca}^{2+}$  waves all represent a cell as a one-dimensional line. From an experimentalist's point of view, this might appear to be a crude approximation to the real cellular shape and morphology. The value of one-dimensional models is their ability to identify key mechanisms of  $\text{Ca}^{2+}$  wave propagation and to provide a thorough mathematical underpinning of the intracellular processes that drive  $\text{Ca}^{2+}$  waves. In turn, this establishes confidence in the chosen modeling framework to explore two- and three-dimensional models while avoiding spurious results.

Coupled with the stochastic description of  $\text{Ca}^{2+}$  release, two-dimensional models of the intracellular  $\text{Ca}^{2+}$  concentration have provided intriguing insights into the generation and propagation of  $\text{Ca}^{2+}$  waves (see Figure 1). For example, a stochastic FDF model exhibits spatially synchronized oscillations, i.e. every point in the cell oscillates with the same phase as its neighbors and the averaged  $\text{Ca}^{2+}$  concentration shows regular oscillations (Coombes and Timofeeva 2003). However, as soon as the random opening of the  $\text{Ca}^{2+}$  releasing channels is turned off, the oscillations disappear. Although the cell-wide signal looks deterministic and homogenous, an ODE framework as discussed above fails to provide the right mechanism. It is the interplay between the spatial arrangement of the  $\text{Ca}^{2+}$  release channels and the fluctuations of channel opening that are important, neither of which are captured by ODEs. Similarly, the results in (Falcke 2003b) suggest that  $\text{Ca}^{2+}$  waves are initiated by the random formation of a critical nucleus. Only if a sufficient number of  $\text{IP}_3\text{R}$  clusters open at the same time in close proximity will a  $\text{Ca}^{2+}$  wave be born. Two-dimensional simulations have been instrumental in providing first estimates for the number of  $\text{IP}_3\text{Rs}$  per cluster, which is still hard to determine experimentally due to the small cluster diameter (Swillens et al. 1999; Shuai and Jung 2003a; 2003b), but see (Smith and Parker 2009) for a recent experimental measurement.

The success of two-dimensional simulations and the availability of more powerful computing facilities have promoted the study of  $\text{Ca}^{2+}$  signals in a three-dimensional cellular environment (Izu et al. 2006; Means et al. 2006; Rüdiger et al. 2007; Li and Holden 2009; Skupin et al. 2010; Solovey et al. 2011; Thurley and Falcke 2011; Thul et al. 2012). Each of these studies focuses on a particular aspect of  $\text{Ca}^{2+}$  signaling such as a realistic distribution of  $\text{Ca}^{2+}$  release sites, ER geometry, or the interaction of a small number of  $\text{IP}_3\text{Rs}$  and  $\text{IP}_3\text{R}$  clusters with detailed gating schemes. Taken together, this research provides a kaleidoscopic view of the nature of intracellular  $\text{Ca}^{2+}$  signals and highlights two of the main characteristics of intracellular  $\text{Ca}^{2+}$  dynamics. Firstly, intracellular  $\text{Ca}^{2+}$  is an intrinsic stochastic medium. The random state transitions that occur at a cluster of  $\text{IP}_3\text{Rs}$  is due to the continuous binding and unbinding of  $\text{Ca}^{2+}$  and  $\text{IP}_3$  to a small number of binding sites and is instrumental in generating  $\text{Ca}^{2+}$  puffs. The large





**Figure 1:**  $\text{Ca}^{2+}$  waves in a stochastic two-dimensional FDF model. The eight panels show snapshots of multiple  $\text{Ca}^{2+}$  waves (time runs from left to right, top to bottom). The first wave (top left) is triggered at the beginning of the simulation, while all other waves emerge spontaneously due to fluctuations of  $\text{Ca}^{2+}$  release. For a three-dimensional stochastic FDF model, I refer the reader to (Thul et al. 2012)

$\text{Ca}^{2+}$  concentrations that occur at an open cluster saturate any deterministic binding dynamics and hence cannot explain experimentally observed puff statistics (Thul and Falcke 2004a; 2004b). As a consequence intracellular  $\text{Ca}^{2+}$  oscillations emerge at the cellular level through the stochastic orchestration of  $\text{Ca}^{2+}$  puffs. Single puff sites do not exhibit oscillatory dynamics (Thurley et al. 2011). Secondly, the true nature of  $\text{Ca}^{2+}$  dynamics can only be captured in spatially extended models. Microdomains and  $\text{Ca}^{2+}$  waves clearly indicate that cells are not well-stirred bioreactors where the  $\text{Ca}^{2+}$  concentration is the same across the entire cell. The generation and molecular read-out of  $\text{Ca}^{2+}$  signals depends on the local environment, not on properties of the bulk  $\text{Ca}^{2+}$  concentration.

## The road ahead

Modeling intracellular  $\text{Ca}^{2+}$  dynamics has already come a long way and has significantly advanced our understanding of this most versatile second messenger. So far, we have gained great insight into individual levels of the  $\text{Ca}^{2+}$  signaling hierarchy, e.g. for  $\text{Ca}^{2+}$  blips, puffs and sparks or whole cell  $\text{Ca}^{2+}$  waves. The challenge for the future is to construct models that span the entire spatio-temporal range (Thurley et al. 2012). For  $\text{IP}_3$  mediated  $\text{Ca}^{2+}$  patterns, this means computational frameworks that take us from the stochastic binding of  $\text{Ca}^{2+}$  and  $\text{IP}_3$  to their respective binding sites on the  $\text{IP}_3\text{R}$ , to a cellular response accounting for the often irregular three-dimensional geometry of cells and the spatially heterogeneous expression of large numbers of  $\text{IP}_3\text{R}$  clusters. The constant advances in computational power will certainly help us to achieve this goal. At the same time, modelers have to improve existing models and to develop novel techniques that characterize intracellular  $\text{Ca}^{2+}$  dynamics more efficiently without sacrificing details of the  $\text{Ca}^{2+}$  signaling toolkit. Constructing models that are firmly rooted in experimental findings and successfully predict experimental results, while at the same time providing mechanistic interpretations of signaling pathways, will serve as a guiding principle for future research both in modeling and experiment.

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